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Incorporation of [U-^{14}C]Glucose into Metabolites of Brain, Liver and Blood of Rats Pretreated with Reserpine or Phenothiazines

Drug-induced Parkinsonism in the rat – A model for biochemical investigation of the Parkinson-syndrome, II

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Summary: Parkinsonism was induced in rats by using phenothiazines (Butyrylperazin and Thioproperazin), (P-group), or reserpine, (R-group). [U-^{14}C)*D*-glucose was administered when the symptoms of Parkinsonism had become fully developed. Concentrations and radioactivities of different metabolites were studied in brain, liver and blood serum.

1. Both types of treatments resulted in a decrease in the synthesis of amino acids from [^{14}C]glucose in the brain. The concentrations of amino acids and the glycogen remained uneffected. Phenothiazines enhanced the conversion of lipids, while reserpine increased their concentration.
2. Reduced de novo synthesis of amino acids was recorded in the liver. Phenothiazines resulted in the storage of glycogen and lipids; reserpine resulted in the storage of lipids and enhanced the conversion of glycogen.
3. Both treatments caused a fall in the amino acid concentration of the blood serum. A rise in the specific radioactivity of blood amino acids was observed in the P-group, while a decrease in specific radioactivity was observed in the R-group. A hyperglycemia was induced in the R-group with reduced specific radioactivity of glucose in both P- and R-groups. A reduction in lipid concentration of blood serum was achieved with an increased specific radioactivity in P-group and decreased radioactivity in R-group.
4. The changes in amino acids common to both treatments are also observed in human Parkinsonism.

Der Einbau von [U-^{14}C]D-Glucose in Metabolite in Hirngewebe, Leber und Blut von Ratten, die mit Reserpin oder mit Phenothiazinen vorbehandelt wurden

Drogeninduzierter Parkinsonismus der Ratte – ein Modell für biochemische Untersuchungen des Parkinsonsyndroms, 2. Mitteilung

Zusammenfassung: In Ratten wurde ein Parkinsonoid durch Behandlung mit Phenothiazinen (Butyrylperazin und Thioproperazin) – P-Gruppe – oder mit Reserpin – R-Gruppe – induziert. Zum Zeitpunkt der vollen Entwicklung des Parkinsonoids wurde [U-^{14}C]*D*-Glucose verabreicht. Konzentrationen und Radioaktivität verschiedener Metabolite wurden in Hirngewebe, Leber und Blutserum untersucht.

1. Beide Behandlungsformen verursachten eine Verminderung der Aminosäuresynthese aus [^{14}C]Glucose im Hirngewebe. Die Konzentration der Summe der Aminosäuren und des Glykogens blieben unverändert. Phenothiazine bewirkten einen verstärkten Umsatz von Lipiden, während Reserpin deren Konzentration erhöhte.
2. In der Leber wurde eine Verminderung der Neusynthese von Aminosäuren gefunden. Außerdem bewirkten Phenothiazine eine Speicherung von Glykogen und Lipiden, Reserpin hingegen eine Speicherung von Lipiden und eine Umsatzerhöhung von Glykogen.

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3. Beide Behandlungsweisen verursachten eine Abnahme der Aminosäurekonzentration im Serum. Es wurde ein Ansteigen der spezifischen Radioaktivität in der P-Gruppe und eine Abnahme in der R-Gruppe gemessen. In der R-Gruppe trat eine Hyperglykämie auf, die spezifische Radioaktivität der Blutglucose war aber in beiden Gruppen vermindert. Die Lipidkonzentration im Serum nahm ebenfalls in beiden Gruppen ab, begleitet von einem Anstieg der spezifischen Radioaktivität in der P-Gruppe und von einer Abnahme in der R-Gruppe.
4. Veränderungen, die durch beide Behandlungsformen induziert wurden: Veränderung im Aminosäuregleichgewicht, die derjenigen vergleichbar war, die beim menschlichen Parkinsonismus auftritt.

Introduction

Parkinson's disease as well as human drug induced Parkinsonism have been reported to exhibit comparable changes in the spectrum of amino acids in cerebrospinal fluid (1). The recorded changes of free amino acids in brains of rats were comparable to those of human CSF, in those cases where the experimental tratment lead to akinesis, rigor and tremor (2). An increase in the concentrations of serine, threonine and glycine and a decreased glutamic acid concentration were observed in human CSF as well as in brains of rats.

Treatment with reserpine has been shown to induce Parkinsonism (6). The preparation of a model that shows drug-induced Parkinsonian symptoms needs very careful treatment, since the effect of the tested drugs on metabolism depends strongly on various parameters: dosage, temperature (3), mode of application (4), duration of treatment (5). It has been reported that a strong cataleptic state produced by phenothiazines is not suitable as a model of Parkinsonism (6).

In the present work, the changes induced in the different metabolites in two parkinsonian models produced by treatment with reserpine or phenothiazines, have been studied. The investigation of whether induced changes in amino acids, important as they are, occur exclusively in the central nervous system is also one of the objectives of our present work.

Materials and Methods

Animals

Wistar rats (150–180 g) kept on a normal diet were used in the present work. The room temperature was kept at 32° C, and the animals showed no signs of hypothermia.

Induction of Parkinsonism

By Reserpine: Treatment lasted for 4 days. Animals were given intraperitoneal injection of 0.3 mg/kg reserpine on the first and the third day and 0.8 mg/kg on the second and the fourth day.

By Phenothiazines: Treatment lasted for 10 days. 2 mg/day butyrylperazin were mixed with bread, and care was exercised to ensure total consumption. On the day 11 animals were injected subcutaneously with 27 mg/kg thioproperazin.

Four hours after application of the last drug, 666 kBq = 18 μCi [U-^{14}C]*D*-glucose (Radiochemical Center Amersham) were injected intravenously. Twenty minutes later, the animals were decapitated.

Preparation of blood, tissues and homogenates

After decapitation, blood was collected and allowed to coagulate, centrifuged and serum was separated and kept in deep freeze. Brain and liver were removed immediatly after decapitation. The brain was dissected on a cooling plate (obtained from the firm of Neumann, München) at −20 °C, according to the method of *Popow* et al (7). All tissues were frozen in dry ice and processed on the same day. The brain was dissected into the following parts:

Cortex region: Neopallium frontal and parietal parts; appr. 20% of the hippocampus.

Striatum region: Putamen; globus pallidus; caput nuclei caudati; commissural plate; fornix.

Thalamus region: Thalamus and hypothalamus; fornix; cauda; nuclei caudati; pedunculi cerebri.

Cerebellum

The remains of the brain after dissection were used for the analysis of glycogen and lipids. Samples of kidney, spleen, heart, gonads, adipose tissue and muscles were prepared, too.

Liver and the 4 brain regions were homogenized in a 10-fold volume of 50 g/l trichloroacetic acid at 0 °C. Homogenates were centrifuged. The sediment was washed twice with distilled water, and washings and supernatants were pooled.

Blood serum was deproteinized by mixing 1 ml serum with 1 ml 0.6 mol/l perchloric acid. The mixture was centrifuged at 3000 rpm. The sediment was washed with 1 ml of the perchloric acid. The wash and the supernatant were pooled and brought to pH 7 by addition of KOH solution. The precipitated salt was removed by centrifugation. The supernatant was brought to a total volume of 3.5 ml.

Measurement of radioactivities

The total radioactivities of different serum and tissue extracts were measured by mixing 0.5 ml of the extract with a *Bray* system (Packard Scintillation counter).

Estimation of ninhydrin reactive compounds and amino acids

An aliquot of each extract was used to measure the ninhydrin-reactive compounds using the method of *Moore & Stein* (8). Another aliquot was placed into ion exchange resin Dowex 50 X 4 at pH 2.0. Amino acids were eluted, dried and dissolved in distilled water. Separate aliquots of the amino acid extract were used for measurement of concentration (8), and for the determination of radioactivity using the *Bray* scintillation mixture.

Estimation of quantity and radioactivity of lipids

The remains of the brains and a sample of liver were weighed and homogenized in a ten fold volume of 100 g/l trichloroacetic acid. Tissue and serum lipids were extracted by a chloroform/methanol mixture (9). The organic phase was separated and

evaporated to dryness under vacuum. Total lipids were estimated gravimetrically. Lipids were then dissolved in the *Bray* system for the estimation of the radioactivity.

Estimation of tissue glycogen and serum glucose

The glycogen content of brain was estimated in the remaining defatted brain, together with the aqueous trichloroacetic acid extract, after lipid extraction. Liver glycogen was estimated on a fresh tissue sample by homogenisation in a ten fold volume of 100 g/l trichloroacetic acid.

Both brain and liver extracts were treated with 600 g/l KOH. Precipitated glycogen was washed with absolute ethanol (A.R.). Glycogen was hydrolyzed with 0.75 mol/l HCl in a boiling water bath. Aliquots were used to measure the glucose content enzymatically using test packs (Calbiochem, Los Angeles). Serum glucose was estimated in deproteinized filtrate using the same method.

Measurements of the radioactivity were undertaken by mixing tissue and serum extracts with twice the volume of 2,4-diphenyl-hydrazine 10 g/l in HCl (10). The products of the reaction were extracted 3 times with a 10-fold volume of ethyl acetate at 0 °C. The extract was evaporated to dryness and radioactivity was measured, using the *Bray* scintillation system.

Results

In the present work, the incorporation of ^{14}C into the different metabolites has been studied in rats pretreated with phenothiazines or reserpine. The distribution of radioactivity in different organs was calculated. Taking the brain total radioactivity as 1.0, the radioactivities ranged from 0.04 in the gonads to 0.32 in the kidney. The liver showed the highest radioactivity (0.95), second to the brain.

The incorporation of radioactivity into amino acids, glycogen and lipids was studied in the brains and livers of rats treated with phenothiazines or reserpine.

Distribution of radioactivity

Brain

1. Brain amino acids: The uptake of radioactivity into amino acids in the brain was studied in different brain regions (tab. 1). All groups showed that most of the radioactivity was, in fact, incorporated into amino acids. Thus, the control animals showed an incorporation which varied from 0.73 in the stratum to 0.99 in the cerebellum.

The treated groups showed reduced total radioactivity in the amino acids. Compared with the controls, total radioactivity in the amino acids in the thalamus was decreased to 0.86 and to 0.75 in the cortex in the P-group. On the other hand, in the R-group, the total radioactivity fraction varied from 0.54 in cerebellum to 0.40 in the striatum.

The incorporation of radioactivity into amino acids of brain regions of treated groups showed a reduction which was more pronounced in the R-group than in the P-group. Conversely, the relative radioactivity of amino

Tab. 1. Distribution of radioactivity in amino acids in brain regions of rats treated with phenothiazines or reserpine. Values are given as $\bar{x} \pm s$. n = 6 (each group)

	Control group		Phenothiazine group			Reserpine group		
	Radioactivity [Bq/g tissue]	Radioactivity fraction amino-acids / homogenate	Radioactivity [Bq/g tissue]	Radioactivity fraction of control	Radioactivity fraction amino-acids / homogenate	Radioactivity [Bq/g tissue]	Radioactivity fraction of control	Radioactivity fraction amino-acids / homogenate
Cortex region	Homogenate 4900 ± 1067	–	3683 ± 967	0.75*	–	2250± 683	0.46*	–
	Amino acids 3833 ± 583	0.78	3167 ± 850	0.83	0.86	2050 ± 1017	0.53**	0.91
Thalamus region	Homogenate 4267 ± 1050	–	3683 ± 483	0.86	–	2000 ± 517	0.47**	–
	Amino acids 3500 ± 900	0.82	2667 ± 383	0.76*	0.72	1483 ± 417	0.42**	0.74
Striatum region	Homogenate 4750 ± 1033	–	3850 ± 767	0.81**	–	1917 ± 450	0.40**	–
	Amino acids 3450 ± 817	0.73	3233 ± 583	0.94	0.84	1400 ± 383	0.41**	0.73
Cerebellum	Homogenate 3750 ± 1117	–	2833 ± 333	0.76*	–	2000 ± 450	0.54**	–
	Amino acids 3717 ± 883	0.99	2450 ± 267	0.66*	0.86	1583 ± 367	0.43**	0.79

Differences from control significant at * $0.05 > P > 0.01$
** $0.01 > P > 0.001$

acids compared to the total homogenate, was increased in the cortex and striatum of treated groups, while a reduction was noted in the thalamus and cerebellum of treated groups.

2. Brain glycogen and lipids: Calculating the average radioactivity in the different brain regions and taking it as 1.0, the glycogen showed 0.0002, while the lipid showed 0.007 radioactivity (tab. 2). Of the treated animals, those in P-group showed increased incorporation of radioactivity in glycogen and lipids. On the other hand, a decreased incorporation was observed in the R-group.

Liver

1. Liver amino acids: A comparatively low level of radioactivity was incorporated in amino acids in the control group. It increased in the P-group and showed a slightly lower value in the R-group (tab. 2). The amount of radioactivity incorporated into amino acids was found to decrease on treatment both with phenothiazines (0.83) or with reserpine (0.55).

2. Liver glycogen and lipids: The relative values of radioactivity in glycogen and lipids in the controls were found to be 0.00004 and 0.004 resp. Treatment with reserpine or phenothiazines increased the incorporation of radioactivity into glycogen and lipids (R-group showed no change in the lipids).

Concentrations and specific radioactivities

Brain

1. Brain amino acids: The amino acid concentration is similar in all brain regions studied in control animals. The specific radioactivity of amino acids can be arranged according to: cortex > cerebellum > striatum > thalamus (tab. 3). Treatment with phenothiazines or reserpine produced a rise in the quantity of amino acids in the cortex, with a lowering of the specific radioactivity; this behaviour was more pronounced in the R- than the P-group. On the other hand, the thalamus, striatum and cerebellum showed a fall in the amino acid concentration and in the specific radioactivity (the striatum, in the R-group only, showed a rise in amino acid concentration). The relation changes in the R-group were greater than in the P-group.

2. Brain glycogen and lipids: Control animals showed higher specific radioactivity for glycogen than for lipids. Treated groups showed a rise in the concentration and in the specific radioactivity of the glycogen. No change has been seen in specific radioactivity in the R-group. A fall was observed in the lipid concentration in P-group, while its specific radioactivity increased. In the reserpine group, a rise in lipid concentration was observed, while the specific radioactivity decreased, indicating enhanced formation of lipids from non carbohydrate sources (tab. 4).

Tab. 2. Uptake of radioactivity into different metabolits in brain and liver of rats treated with phenothiazines or reserpine. Values are given as $\bar{x} \pm s$. n = 6 (each group)

	Control group		Phenothiazine group			Reserpine group		
	Radioactivity [Bq/g tissue]	Radioactivity fraction of homogenate	Radioactivity [Bq/g tissue]	Radioactivity fraction of control	Radioactivity fraction of homogenate	Radioactivity [Bq/g tissue]	Radioactivity fraction of control	Radioactivity fraction of homogenate
Brain	Homogenate+							
	4425 ± 918	–	3513 ± 824	0.79*	–	2042 ± 880	0.46*	–
	Glycogen							
	1.0 ± 0.17	0.0002	1.17 ± 0.33	1.17	0.0003	1.0 ± 0.17	0.00	0.0005
	Lipids							
	30 ± 6.67	0.0068	36.7 ± 5.0	1.22	0.0105	20 ± 8.3	0.67	0.0082
Liver	Homogenate							
	4233 ± 967	–	2600 ± 550	0.61*	–	3250 ± 583	0.77**	–
	Amino acids							
	333 ± 80	0.079	278 ± 65	0.83**	0.11	183 ± 75	0.55	0.056
	Glycogen							
	0.17 ± 0.033	0.00004	0.83 ± 0.017	4.00**	0.0003	0.33 ± 0.017	2.00*	0.001
	Lipids							
	1.5 ± 6.67	0.0035	75 ± 13.3	40.0**	0.029	15 ± 3.3	10.0*	0.0046

+ ... average of observations of different remaining brain regions (see methods)
Differences from control significant at * $0.05 > P > 0.01$
** $0.01 > P > 0.001$

Tab. 3. Changes in the concentration and specific radioactivity of amino acids (AA) of different brain regions in rats treated with phenothiazines or reserpine.
Values are given as $\bar{x} \pm$ s. n = 6 (each group)

	Control group	Phenothiazine group		Reserpine group	
	Concentration [mg AA/g tissue] resp. Radioactivity [Bq/g AA]	Concentration [mg AA/g tissue] resp. Radioactivity [Bq/g AA]	Concentration fraction of control resp. Radioactivity fraction of control	Concentration [mg AA/g tissue] resp. Radioactivity [Bq/g AA]	Concentration fraction of control resp. Radioactivity fraction of control
Cortex region	4.4 ± 0.7 867 ± 83	5.2 ± 0.5 567 ± 167	1.18* 0.65**	5.8 ± 0.4 350 ± 133	1.32** 0.40**
Thalamus region	4.7 ± 0.7 667 ± 183	4.6 ± 0.9 583 ± 183	0.98 0.88	3.9 ± 1.6 250 ± 50	0.83** 0.38**
Striatum region	4.8 ± 1.4 717 ± 100	4.5 ± 1.3 533 ± 133	0.94 0.74	5.1 ± 0.7 267 ± 117	1.06 0.37**
Cerebellum	4.8 ± 1.0 767 ± 283	4.5 ± 1.3 550 ± 133	0.94 0.72	4.6 ± 1.8 333 ± 200	0.96 0.43**

Differences from control significant at * $0.05 > P > 0.01$
** $0.01 > P > 0.001$

Tab. 4. Incorporation of radioactivity into glycogen (G) and lipids (L) in brains of rats treated with phenothiazines or reserpine.
Values are given as $\bar{x} \pm$ s. n = 6 (each group)

	Control group	Phenothiazine group		Reserpine group	
	Concentration [mg/g tissue] resp. Radioactivity [Bq/mg G resp. L]	Concentration [mg/g tissue] resp. Radioactivity [Bq/mg G resp. L]	Concentration fraction of control resp. Radioactivity fraction of control	Concentration [mg/g tissue] resp. Radioactivity [Bq/mg G resp. L]	Concentration fraction of control resp. Radioactivity fraction of control
Glycogen	1.9 ± 0.5 2.33 ± 0.67	2.1 ± 0.2 2.83 ± 0.67	1.10 1.21	2.2 ± 0.1 2.33 ± 0.67	1.16 1.00
Lipids	327 ± 12 0.067 ± 0.017	195 ± 57 0.133 ± 0.034	0.60** 2.00*	371 ± 26 0.050 ± 0.017	1.13** 0.75

Difference from control significant at * $0.05 > P > 0.01$
** $0.01 > P > 0.001$

Liver

Highest specific radioactivity was shown by the amino acids, followed by glycogen, then lipids (tab. 5). The P-group showed a rise in amino acid concentration (1.95) and a fall in its specific radioactivity (0.43). This may indicate enhanced formation of amino acids from non-carbohydrate sources, and depressed incorporation of [^{14}C]glucose. On the other hand, the R-group showed a fall in amino acid concentration (0.74) and a fall in its specific radioactivity (0.74), indicating that the drug depressed amino acid synthesis from injected ^{14}C.

The P-group showed a rise in the concentration and specific activity of glycogen, indicating enhanced glycogen synthesis from injected ^{14}C. Conversely, R-group animals showed a fall in the level of glycogen and a rise in its specific activity. This may indicate increased incorporation of ^{14}C into liver glycogen from injected glucose.

A rise was observed in the concentration and specific radioactivity of lipids in both P- and R-groups. The effect in P-group was greater than in R-group. This result may indicate de novo synthesis of lipids from labelled glucose, stimulated by phenothiazines and reserpine.

Blood serum

The results of analysis of blood serum are given in table 6. The treatment with phenothiazines and reserpine produced a fall in total amino acids, the decrease being greater in the P-group. The specific radioactivity increased in the P-group and decreased in the R-group, the former indicating enhanced incorporation of radioactivity into amino acids.

A state of hyperglycemia was observed, and this was pronounced in the R-group. On the other hand, a decreased specific radioactivity of blood glucose was observed in both treated groups, being greater in the

Tab. 5. Incorporation of radioactivity into the amino acids (AA), glycogen (G) and lipids (L) in the livers of rats treated with phenothiazines or reserpine. Values are given as $\bar{x} \pm s$. n = 6 (each group)

	Control group	Phenothiazine group		Reserpine group	
	Concentration [mg/g tissue] resp. Radioactivity [Bq/mg AA resp. G resp. L]	Concentration [mg/g tissue] resp. Radioactivity [Bq/mg AA resp. G resp. L]	Concentration fraction of control resp. Radioactivity fraction of control	Concentration [mg/g tissue] resp. Radioactivity [Bq/mg AA resp. G resp. L]	Concentration fraction of control resp. Radioactivity fraction of control
Amino acids	3.8 ± 0.3	7.4 ± 1.0	1.95	2.8 ± 0.7	0.74
	88.3 ± 35	38.3 ± 10	0.43*	65 ± 18.3	0.74**
Glycogen	10.1 ± 2.2	13.5 ± 3.4	1.34*	3.7 ± 0.6	0.37**
	0.16 ± 0.013	0.30 ± 0.038	1.91**	0.46 ± 0.13	2.94**
Lipids	119 ± 24	240 ± 32	2.02**	180 ± 15	1.51**
	0.07 ± 0.028	0.33 ± 0.028	4.60**	0.09 ± 0.08	1.26

Differences from control significant at * $0.05 > P > 0.01$
** $0.01 > P > 0.001$

Tab. 6. Concentration and specific radioactivity of amino acids (AA), glucose (glc) and lipids (L) in the blood serum of rats treated with phenothiazines or reserpine. Values are given as $\bar{x} \pm s$. n = 6 (each group)

	Control group	Phenothiazine group		Reserpine group	
	Concentration [g/l] resp. Radioactivity [Bq/g AA resp. glc resp. L]	Concentration [g/l] resp. Radioactivity [Bq/g AA resp. glc resp. L]	Concentration fraction of control resp. Radioactivity fraction of control	Concentration [g/l] resp. Radioactivity [Bq/g AA resp. glc resp. L]	Concentration fraction of control resp. Radioactivity fraction of control
Amino acids	1.28 ± 0.30	0.70 ± 0.06	0.55**	0.95 ± 0.16	0.74
	0.023 ± 0.003	0.045 ± 0.006	1.93**	0.020 ± 0.0015	0.86
Glucose	1.29 ± 0.18	1.31 ± 0.17	1.02	1.53 ± 0.21	1.19
	0.300 ± 0.020	0.077 ± 0.035	0.26**	0.167 ± 0.052	0.56**
Lipids	4.40 ± 0.94	2.70 ± 0.56	0.62**	2.60 ± 0.39	0.59**
	0.0012 ± 0.00017	0.0018 ± 0.00017	1.57	0.0007 ± 0.0003	0.57

Differences from control significant at * $0.05 > P > 0.01$
** $0.01 > P > 0.001$

P-group. This indicates the mobilisation of non-labelled glycogen, and there was, in fact, a lowering of liver glycogen in the R-group. The fall in the specific radioactivity of serum glucose may explain the increased specific radioactivity incorporated into liver and brain amino acids, and glycogen.

A fall in the concentration of serum lipids was observed in both P- and R-groups. A rise was observed in the specific radioactivity of serum lipids in the P-group, while in the R-group they showed a decrease in specific radioactivity.

Discussion

In *Parkinson'* disease as well as in drug induced Parkinsonism in man and animals, previous reports indicate identical alterations of the amino acid spectrum both in CSF or CNS (11). The present study reports the results of the change occurring in the metabolism of amino acids, carbohydrates and lipids in two models of drug induced Parkinsonism in the rat.

In the phenothiazine-treated animals, the specific radioactivity of amino acids decreased in the brain, while their concentration remained nearly unaffected. This may be explained in the light of previous work (1, 2), in which a decrease observed in glutamic acid was compensated by an augmentation of glycine, serine and related amino acids. There is a correlation between glucose utilisation and glutamic acid level in the CNS; thus, the disturbance of glucose metabolism was reported to produce a decrease in the glutamic acid level in the CNS (12). The reported simultaneous increase in serine and glycine (1, 2) occurs only in extrapyramidal syndromes (11).

Neuroleptics of the phenothiazine type are still studied in connection with their effects on *L*-DOPA metabolism (13–21). These drugs are postulated to provoke extrapyramidal disorders of movements by blocking the dopaminergic receptors (22). It appears that this is not the only cause of phenothiazine induced Parkinsonism. It appears that the disturbances in the amino acids glutamic acid and γ-aminobutyric acid may have additional influence on the balance of neurotransmitters (2).

It is found in the present work that the disturbances recorded in the brain amino acids are quite different from those in the liver. The present data show that liver exhibited a marked increase in the amino acid concentration but not in its specific radioactivity. This can be attributed to the catabolism of protein or the biosynthesis of amino acids from non carbohydrate substances.

Hyperglycemia was not recorded, but it has been reported (4, 23) that hyperglycemia is produced by chlorpromazin. In the present investigation, there appeared to be an increase in the uptake of glucose, as evidenced by the decrease in the specific radioactivity of blood glucose. Moreover, tissues appear to exhibit accelerated glucose catabolism under the conditions of the present work. This view is supported by the low incorporation of ^{14}C into tissue glycogen.

The glycogen content in brain tissue was found to be unchanged. Similar findings have been reported following the application of chlorpromazin (24). The concentration and the specific radioactivity of liver glycogen was increased due to de novo synthesis. Moreover, glycogenolysis may be reduced. *Rasmussen* (25) noted that chlorpromazin in vitro impedes the epinephrine-controlled glycogen mobilisation by suppressed cAMP production.

Like the phenothiazines, *reserpine* can also produce akinesis, tremor and α-rigidity in men and in rats (26).

The monoamine storing vesicles become depleted in brain and in peripheral tissues (27–31), which might influence endocrine regulation (32). In our experiments, specific radioactivity of amino acids decreased, but the concentration remained unchanged. This is in accordance with the effect of phenothiazines.

Reserpine as well as phenothiazines lowered the incorporation of ^{14}C from [U-^{14}C]glucose in the brain and in the liver of rats, but the effect of reserpine was less pronounced. In the present work the drug caused a hyperglycemia, as reported earlier (33). This could be due to a mobilisation of glycogen, since the content of glycogen in liver and the specific radioactivity of glucose in blood serum were both diminished. The increased specific radioactivity of liver glycogen indicates an enhanced turnover. The present findings seem to be in contrast to the observations of other authors such as *Balzer* et al (34) and *Albrecht* (35), who found an increased glycogen content in liver after reserpine treatment. They interpreted their results by an enhanced glycogenesis and simultaneous of phosphorylase. This explanation is consistent with our findings, because the glycogen content of the liver depends on dosage and on the mode of application of reserpine. Under different experimental conditions phosphorylase is activated to different extents (31, 34, 36).

In brain as well as in liver after reserpine application we found an increase in lipids which are not synthesized from [^{14}C]glucose, whereas the treatment of rats by phenothiazines, as performed in our laboratory, led to an enhanced specific radioactivity of lipids.

The most important result of our investigation is the fact that in the two models of Parkinsonism the only alterations common to both are found in the amino acid metabolism of the brain. Data concerning the individual amino acids have been published previously (38, 39).

References

1. Bruck, H., Gerstenbrand, F., Gnad, H., Gründig, E. & Prosenz, P. (1966), Psychiatr. Neurol. (Basel), *151*, 81–87.
2. Gründig, E. & Hanbauer, I. (1970), J. Neurochem. *17*, 215–220.
3. Mueller, P. S., McDonald, R. K. & Sribney, M. (1967), Proc. Soc. Exp. Biol. Med. (N.Y.) *126*, 339.
4. Himwich, W. A. & Davis, J. M. (1972), Biol. Psychiatry *5/1*, 89–94.
5. Lafrance, L. & Le Blanc, J. (1967), Can. J. Physiol. Pharmacol. *45*, 215–224.
6. Hanbauer, I. (1968), Diss. Univ. Wien.
7. Popov, N., Pohle, W. Rosler, V. & Matthies, H. (1967), Acta Biol. Med. Ger. *18*, 695–702.
8. Moore, S. & Stein, H. W. (1948), J. Biol. Chem. *176*, 367–388.
9. Folch, J., Lees, M., Sloane, L. & Stanley, G. M. (1957), J. Biol. Chem. *226*, 497–509.
10. Neuberg, C. & Strauss, E. (1946), Arch. Biochem. *11*, 457–463.
11. Gründig, E. & Gerstenbrand, F. (1970), Wien. Klin. Wochenschr. *82*, 811–816.
12. Gründig, E., Salvemoser, F. & Bretschneider, R. (1963), Z. Ges. Exp. Med. *137*, 94–107.
13. Holzer, G. & Hornykiewicz, O. (1959), Arch. Exp. Pathol. Pharmacol. *237*, 27–33.
14. Anden, N. E. & Roos, B. E. (1964), Life Sci. *3*, 149–158.
15. Laverty, R. & Sharman, D. F. (1965), Br. J. Pharmacol. *24*, 759–772.
16. Bernheimer, H., Hornykiewicz, O. (1965), Arch. Exp. Pathol. Pharmakol. 251–253.
17. Pletscher, A. & Da Prada, M. (1966), Helv. Physiol. Pharmacol. Acta *24*, C45–C47.
18. Burkhard, W. P., Gey, K. F. & Pletscher, A. (1967), Nature (London) *213*, 732–733.

19. Snyders, S. H. (1967), Am. J. Orthopsychiatry *37*, 864–879.
20. Nyback, H. & Sedvall, G. (1968), J. Pharmacol. Exp. Ther. *162*, 294–301.
21. Bartholini, G. & Pletscher, A. (1969), Experientia *25*, 919–920.
22. Carlsson, A. & Lindqvist, M. (1963), Acta Pharmacol. Toxicol. *20*, 140–144.
23. Jeske, J. & Stochla, K. (1966), Farm. Pol. *22*, 653–659.
24. Fujita, T. (1967), Kobe J. Med. Sci. *13* (1) 39–45.
25. Rasmussen, H. (1971), 1*st* Am. meeting Soc. for Neuroscience, Washington D.C. 27–30 Oct. 1971 (see l.c. (5)).
26. Balzer, H., Holtz, P. & Palm, D. (1961), Experientia *17*, 304–305.
27. Shore, P. A., Pletscher, A., Tomwich, E. C., Carlsson, A., Knutzmann, R. & Brodie, B. B. (1957), Ann. N.Y. Acad. Sci. *66*, 609–615.
28. Shore, P. A. (1962), Pharmacol. Rev. *14*, 531–550.
29. Hassler, R. & Bak, I. J. (1966), Nervenarzt *37*, 493–498.
30. Anden, N. E. (1967), Eur. J. Pharmacol. *1*, 1–5.
31. Johnson, G., Kim, E. G., Veldkamp, W. & Russel, R. (1967), Biochem. Pharmacol. *16*, 401–403.
32. Bein, H. J. (1956), Pharmacol. Rev. *8*, 435–453.
33. Kuschke, H. J. & Frantz, J. (1955), Arch. Exp. Pathol. Pharmacol. *224*, 269–274.
34. Balzer, H., Holtz, P. & Palm, D. (1961), Experientia *17*, 304–305.
35. Albrecht, W. (1957), Klin. Wochenschr. *35*, 588–590.
36. Estler, J. C. (1965), Arch. Exp. Pathol. Pharmakol. *252*, 63–67.
37. Conway, E. J.; O'Malley, E. (1942), Biochem. J. *36*, 655–661.
38. Gründig, E., Abdel Raheem, K., Salvenmoser, F. & Kollner, U. (1975), IInd Czechoslovak Congress with International Participation on Clinical Biochemistry (September 17–19, 1975, Prague).
39. Gründig, E., Abdel Raheen, K. Salvenmoser, F., Schedel, R. & Weiss, J. (1976), J. Psychopharmacol. *47*, 111–118.

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